

SEQUENCE LISTING

<110> Leppla, Stephen H.
Avallone, Jennifer
Bugge, Thomas
Liu, Shi-Hui
Osorio, Manuel
The Government of the United States of America
as represented by The Secretary of the
Department of Health and Human Services

<120> Activation of Recombinant Diphtheria Toxin Fusion
Proteins by Specific Proteases Highly Expressed on the
Surface of Tumor Cells

<130> 015280-478100PC

<140> WO PCT/US04/14306

<141> 2004-05-06

<150> US 60/468,577

<151> 2003-05-06

<160> 30

<170> PatentIn Ver. 2.1

<210> 1

<211> 1683

<212> DNA

<213> Corynebacterium diphtheriae

<220>

<223> wild type Diphtheria toxin (DT)

<220>

<221> sig_peptide

<222> (1)..(75)

<223> signal sequence

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<210> 2
<211> 1566
<212> DNA
<213> Artificial Sequence

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<220>
<223> Description of Artificial Sequence:DTGM-L1
      DT-GMCSF fusion protein in which native furin
      recognition cleavage site replaced by matrix
      metalloproteinase (MMP) recognition cleavage site

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1566

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<210> 3
<211> 1566
<212> DNA
<213> Artificial Sequence

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<220>

<223> Description of Artificial Sequence:DTGM-L2
DT-GMCSF fusion protein in which native furin
recognition cleavage site replaced by matrix
metalloproteinase (MMP) recognition cleavage site

<400> 3

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<210> 4

<211> 1560

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:DTGM-U2 DT-GMCSF fusion
protein in which native furin recognition cleavage site
replaced by urokinase-type plasminogen activator (uPA)
recognition cleavage site

<400> 4

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<210> 5

<211> 1560

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:DTGM-U3 DT-GMCSF fusion protein in which native furin recognition cleavage site replaced by urokinase-type plasminogen activator (uPA) recognition cleavage site

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<210> 6

<211> 1341

<212> DNA

<213> Artificial Sequence

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<223> Description of Artificial Sequence:DTEGF-L1 DT-EGF
fusion protein in which native furin recognition
cleavage site replaced by matrix metalloproteinase
(MMP) recognition cleavage site

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<210> 7

<211> 1341

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:DTEGF-L2 DT-EGF
fusion protein in which native furin recognition
cleavage site replaced by matrix metalloproteinase
(MMP) recognition cleavage site

<400> 7

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<210> 8

<211> 1335

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:DTEGF-U2 DT-EGF fusion protein in which native furin recognition cleavage site replaced by urokinase-type plasminogen activator (uPA) recognition cleavage site

<400> 8

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<210> 9

<211> 1335

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:DTEGF-U3 DT-EGF fusion protein in which native furin recognition cleavage site replaced by urokinase-type plasminogen activator (uPA) recognition cleavage site

<400> 9

```

atgggcgcgc acgacgtcgt cgactcttct aaatcttttg tgatggaaaa cttttcttcg 60
taccacggga ctaaacctgg ttatgtagat tccattcaaa aaggatataca aaagccaaaa 120
tctggtacac aaggaaatta tgacgatgat tggaaagggt tttatagtac cgacaataaa 180
tacgacgctg cgggatactc tgtagataat gaaaacccgc tctctggaaa agctggaggc 240

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gtggtcaaag	tgacgtatcc	aggactgacg	aaggttctcg	cactaaaagt	ggataatgcc	300
gaaactatta	agaaagagtt	aggtttaagt	ctcactgaac	cgttgatgga	gcaagtcgga	360
acggaagagt	ttatcaaaaag	gttcggtgat	ggtgcttcgc	gtgtagtgct	cagccttccc	420
ttcgctgagg	ggagttctag	cgttgaatat	attaataact	gggaacaggc	gaaagcgta	480
agcgtagaac	ttgagattaa	ttttgaaacc	cgtggaaaac	gtggccaaga	tgcatgtat	540
gagtatatgg	ctcaagcctg	tgcaaggaaat	ggaagtggaa	aatcagcagg	tagctcattg	600
tcatgcataa	atcttgattg	ggatgtcata	agggataaaa	ctaagacaaa	gatagagtct	660
ttgaaagagc	atggccctat	caaaaataaaa	atgagcgaaa	gtcccaataa	aacagtatct	720
gaggaaaaag	ctaaacaata	cctagaagaa	tttcatcaaa	cggcattaga	gcatcctgaa	780
ttgtcagaac	ttaaaaccgt	tactgggacc	aatcctgtat	tcgctggggc	taactatgcg	840
gcgtgggcag	taaacgttgc	gcaagttatc	gatagcgaaa	cagctgataa	tttggaaaag	900
acaactgctg	ctcttttcgat	acttcctggt	atcggtagcg	taatgggcat	tgcaagcggg	960
gccgttcacc	acaatacaga	agagatagtg	gcacaatcaa	tagctttatc	gtctttaatg	1020
gttgctcaag	ctattccatt	ggtaggagag	ctagttgata	ttggtttcgc	tgcatataat	1080
ttttagagaga	gtattatcaa	tttatttcaa	gtagttcata	attcgtataa	tcgtcccgcg	1140
tattctcccg	ggcataaaaac	gaggcctcat	atgaattccg	atagcgagtg	tcctctgagt	1200
cacgatgggt	actgtctaca	tgacggcgct	tgtatgtata	ttgaggctct	agacaagtac	1260
gcgtgtaatt	gcgttggttg	ctacatcggt	gagcgctgtc	agtatcgaga	tctgaaatgg	1320
tggaactta	gataa					1335

<210> 10

<211> 1581

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:DTIL2-L1 DT-IL2
fusion protein in which native furin recognition
cleavage site replaced by matrix metalloproteinase
(MMP) recognition cleavage site

<400> 10

atgggcgccg	acgacgtcgt	cgactcttct	aaatcttttg	tgatggaaaa	cttttcttcg	60
taccacggga	ctaaacctgg	ttatgtagat	tccattcaaa	aaggatataa	aaagccaaaa	120
tctggtacac	aaggaaatta	tgacgatgat	tggaaagggt	tttatagtag	cgacaataaa	180
tacgacgctg	cgggatactc	tgtagataat	gaaaaccgcg	tctctggaaa	agctggaggc	240
gtggtcaaag	tgacgtatcc	aggactgacg	aaggttctcg	cactaaaagt	ggataatgcc	300
gaaactatta	agaaagagtt	aggtttaagt	ctcactgaac	cgttgatgga	gcaagtcgga	360
acggaagagt	ttatcaaaaag	gttcggtgat	ggtgcttcgc	gtgtagtgct	cagccttccc	420
ttcgctgagg	ggagttctag	cgttgaatat	attaataact	gggaacaggc	gaaagcgta	480
agcgtagaac	ttgagattaa	ttttgaaacc	cgtggaaaac	gtggccaaga	tgcatgtat	540
gagtatatgg	ctcaagcctg	tgcaaggaaat	ggaccattag	gaatggtgag	tcaaggtagc	600
tcattgtcat	gcataaatct	tgattgggat	gtcataaggg	ataaaaacta	gacaaagata	660
gagtctttga	aagagcatgg	ccctatcaaa	aataaaaatga	gcgaaagtcc	caataaaaaca	720
gtatctgagg	aaaaagctaa	acaataccta	gaagaatttc	atcaaaccgc	attagagcat	780
cctgaattgt	cagaacttaa	aaccgttact	gggaccaatc	ctgtattcgc	tggggctaac	840
tatgcggcgt	gggcagtaaa	cgttgcgcaa	gttatcgata	gcgaaacagc	tgataatttg	900
gaaaagacaa	ctgctgctct	ttcgataact	cctgggtatc	gtagcgtaat	gggcattgca	960
gacggtgccc	ttcaccacaa	tacagaagag	atagtggcac	aatcaatagc	tttatcgtct	1020
ttaatggttg	ctcaagctat	tccattggta	ggagagctag	ttgatattgg	tttcgctgca	1080
tataattttg	tagagagtag	tatcaattta	tttcaagtag	ttcataattc	gtataatcgt	1140
cccgcgtatt	ctcccgggca	taaaacgagg	cctcatattg	cacctacttc	aagttctaca	1200
aagaaaacac	agctacaact	ggagcattta	ctgctggatt	tacagatgat	tttgaatgga	1260
attaataatt	acaagaatcc	caaactcacc	aggatgctca	cattttaagtt	ttcatgccc	1320
aagaaggcca	cagaactgaa	acatcttcag	tgtctagaag	aagaactcaa	acctctggag	1380
gaagtgctaa	atthagctca	aagcaaaaac	tttacttaa	gacccaggga	cttaatcagc	1440
aatatcaacg	taatagttct	ggaactaaag	ggatctgaaa	caacattcat	gtgtgaatat	1500
gctgatgaga	cagcaaccat	tgtagaattt	ctgaacagat	ggattacctt	ttgtcaaagc	1560
atcatctcaa	cactgacttg	a				1581

<210> 11
 <211> 1581
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence:DTIL2-L2 DT-IL2
 fusion protein in which native furin recognition
 cleavage site replaced by matrix metalloproteinase
 (MMP) recognition cleavage site

<400> 11
 atgggcgccg acgacgtcgt cgactcttct aaatcttttg tgatggaaaa cttttcttcg 60
 taccacggga ctaaacctgg ttatgtagat tccattcaaa aaggtataca aaagccaaaa 120
 tctggtacac aaggaaatta tgacgatgat tggaaagggt tttatagtag cgacaataaa 180
 tacgacgctg cgggatactc tgtagataat gaaaaccgcg tctctggaaa agctggaggc 240
 gtgggtcaaag tgacgtatcc aggactgacg aagggttctcg cactaaaagt ggataatgcc 300
 gaaactatta agaaagagtt aggtttaagt ctactgaac cgttgatgga gcaagtcgga 360
 acggaagagt ttatcaaaaag gttcgggtgat ggtgcttcgc gtgtagtgct cagccttccc 420
 ttcgctgagg ggagttctag cgttgaatat attaataact gggaacaggc gaaagcgta 480
 agcgtagaac ttgagattaa ttttgaaacc cgtggaaaac gtggccaaga tgcgatgtat 540
 gagtatatgg ctcaagcctg tgcaggaaat ggaccattag gattatgggc acaaggtagc 600
 tcattgtcat gcataaatct tgattgggat gtcataaggg ataaaactaa gacaaagata 660
 gagtctttga aagagcatgg ccctatcaaa aataaaatga gcgaaagtcc caataaaaca 720
 gtatctgagg aaaaagctaa acaataccta gaagaatttc atcaaacggc attagagcat 780
 cctgaattgt cagaacttaa aaccgttact gggaccaatc ctgtattcgc tggggctaac 840
 tatgcgcgct gggcagtaaa cgttgcgcaa gttatcgata gcgaaacagc tgataatttg 900
 gaaaagacaa ctgctgctct ttcgatactt cctgggtatcg gtagcgtaat gggcattgca 960
 gacggtgccg ttcaccacaa tacagaagag atagtggcac aatcaatagc tttatcgtct 1020
 ttaatggttg ctcaagctat tccattggta ggagagctag ttgatattgg tttcgctgca 1080
 tataattttg tagagagtat tatcaattta tttcaagtag ttcataattc gtataatcgt 1140
 cccgcgtatt ctcccgggca taaaacgagg cctcatatgg cacctacttc aagttctaca 1200
 aagaaaacac agctacaact ggagcattta ctgctggatt tacagatgat tttgaatgga 1260
 attaataatt acaagaatcc caaactcacc aggatgctca catttaagtt ttacatgcc 1320
 aagaaggcca cagaactgaa acatcttcag tgtctagaag aagaactcaa acctctggag 1380
 gaagtgctaa atttagctca aagcaaaaac tttcacttaa gaccagggga cttaatcagc 1440
 aatatcaacg taatagttct ggaactaaag ggatctgaaa caacattcat gtgtgaatat 1500
 gctgatgaga cagcaaccat tgtagaattt ctgaacagat ggattacctt ttgtcaaagc 1560
 atcatctcaa cactgacttg a 1581

<210> 12
 <211> 1575
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence:DTIL2-U2 DT-IL2 fusion
 protein in which native furin recognition cleavage site
 replaced by urokinase-type plasminogen activator (uPA)
 recognition cleavage site

<400> 12
 atgggcgccg acgacgtcgt cgactcttct aaatcttttg tgatggaaaa cttttcttcg 60
 taccacggga ctaaacctgg ttatgtagat tccattcaaa aaggtataca aaagccaaaa 120
 tctggtacac aaggaaatta tgacgatgat tggaaagggt tttatagtag cgacaataaa 180
 tacgacgctg cgggatactc tgtagataat gaaaaccgcg tctctggaaa agctggaggc 240
 gtgggtcaaag tgacgtatcc aggactgacg aagggttctcg cactaaaagt ggataatgcc 300
 gaaactatta agaaagagtt aggtttaagt ctactgaac cgttgatgga gcaagtcgga 360
 acggaagagt ttatcaaaaag gttcgggtgat ggtgcttcgc gtgtagtgct cagccttccc 420
 ttcgctgagg ggagttctag cgttgaatat attaataact gggaacaggc gaaagcgta 480

agcgtagaac	ttgagattaa	ttttgaaacc	cgtggaaaaac	gtggccaaga	tgcgatgtat	540
gagtatatgg	ctcaagcctg	tgcaggaaat	ggaagtggaa	gatcagcagg	tagctcattg	600
tcatgcataa	atcttgattg	ggatgtcata	agggataaaa	ctaagacaaa	gatagagtct	660
ttgaaagagc	atggccctat	caaaaataaa	atgagcgaaa	gtcccaataa	aacagtatct	720
gaggaaaaag	ctaaacaata	cctagaagaa	tttcatcaaa	cggcattaga	gcacccctgaa	780
ttgtcagaac	ttaaaaccgt	tactgggacc	aatcctgtat	tcgctggggc	taactatgcg	840
gcgtgggcag	taaacgttgc	gcaagttatc	gatagcgaaa	cagctgataa	tttggaaaag	900
acaactgctg	ctctttcgat	acttcctggg	atcggtagcg	taatgggcat	tgcagacggt	960
gccgttcacc	acaatacaga	agagatagtg	gcacaatcaa	tagctttatc	gtctttaatg	1020
gttgctcaag	ctattccatt	ggtaggagag	ctagttgata	ttggtttcgc	tgcataataat	1080
ttttagagaga	gtattatcaa	tttatttcaa	gtagttcata	attcgtataa	tcgtcccgcg	1140
tattctccccg	ggcataaaaac	gaggcctcat	atggcaccta	cttcaagttc	tacaaagaaa	1200
acacagctac	aactggagca	tttactgctg	gatttacaga	tgattttgaa	tggaattaat	1260
aattacaaga	atcccaaact	caccaggatg	ctcacattta	agttttacat	gccaagaag	1320
gccacagaac	tgaacatct	tcagtgtcta	gaagaagaac	tcaaacctct	ggaggaagtg	1380
ctaaatttag	ctcaaagcaa	aaactttcac	ttaagacca	gggacttaat	cagcaatata	1440
aacgtaatag	ttctggaact	aaagggatct	gaaacaacat	tcattgtgtga	atatgctgat	1500
gagacagcaa	ccattgtaga	atttctgaac	agatggatta	ccttttgtca	aagcatcatc	1560
tcaacactga	cttga					1575

<210> 13

<211> 1575

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:DTIL2-U3 DT-IL2 fusion protein in which native furin recognition cleavage site replaced by urokinase-type plasminogen activator (uPA) recognition cleavage site

<400> 13

atgggcgccg	acgacgctgt	cgactcttct	aaatcttttg	tgatggaaaa	cttttcttcg	60
taccacggga	ctaaacctg	ttatgtagat	tccattcaaa	aaggatataca	aaagccaaaa	120
tctggtacac	aaggaaatta	tgacgatgat	tggaaagggt	tttatagtac	cgacaataaa	180
tacgacgctg	cgggatactc	tgtagataat	gaaaaccgcg	tctctggaaa	agctggaggc	240
gtggtcaaag	tgacgtatcc	aggactgacg	aaggttctcg	cactaaaagt	ggataatgcc	300
gaaactatta	agaaagaggt	aggtttaagt	ctcactgaac	cgttgatgga	gcaagtcgga	360
acggaagagt	ttatcaaaaag	gttcggtgat	ggtgcttcgc	gtgtagtgct	cagccttccc	420
ttcgctgagg	ggagttctag	cgttgaatat	attaataact	gggaacaggc	gaaagcgta	480
agcgtagaac	ttgagattaa	ttttgaaacc	cgtggaaaaac	gtggccaaga	tgcgatgtat	540
gagtatatgg	ctcaagcctg	tgcaggaaat	ggaagtggaa	aatcagcagg	tagctcattg	600
tcatgcataa	atcttgattg	ggatgtcata	agggataaaa	ctaagacaaa	gatagagtct	660
ttgaaagagc	atggccctat	caaaaataaa	atgagcgaaa	gtcccaataa	aacagtatct	720
gaggaaaaag	ctaaacaata	cctagaagaa	tttcatcaaa	cggcattaga	gcacccctgaa	780
ttgtcagaac	ttaaaaccgt	tactgggacc	aatcctgtat	tcgctggggc	taactatgcg	840
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acaactgctg	ctctttcgat	acttcctggg	atcggtagcg	taatgggcat	tgcagacggt	960
gccgttcacc	acaatacaga	agagatagtg	gcacaatcaa	tagctttatc	gtctttaatg	1020
gttgctcaag	ctattccatt	ggtaggagag	ctagttgata	ttggtttcgc	tgcataataat	1080
ttttagagaga	gtattatcaa	tttatttcaa	gtagttcata	attcgtataa	tcgtcccgcg	1140
tattctccccg	ggcataaaaac	gaggcctcat	atggcaccta	cttcaagttc	tacaaagaaa	1200
acacagctac	aactggagca	tttactgctg	gatttacaga	tgattttgaa	tggaattaat	1260
aattacaaga	atcccaaact	caccaggatg	ctcacattta	agttttacat	gccaagaag	1320
gccacagaac	tgaacatct	tcagtgtcta	gaagaagaac	tcaaacctct	ggaggaagtg	1380
ctaaatttag	ctcaaagcaa	aaactttcac	ttaagacca	gggacttaat	cagcaatata	1440
aacgtaatag	ttctggaact	aaagggatct	gaaacaacat	tcattgtgtga	atatgctgat	1500
gagacagcaa	ccattgtaga	atttctgaac	agatggatta	ccttttgtca	aagcatcatc	1560
tcaacactga	cttga					1575

<210> 14
<211> 22
<212> DNA
<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:universal 5' T7
promoter primer (5' primer for DT constructs)

<400> 14
gtaatacgac tcactatagg gc

22

<210> 15
<211> 61
<212> DNA
<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:U2 3' mutagenic
PCR primer for U2 constructs

<400> 15
gatttatgca tgacaatgag ctacctgctg atcttccact tccatttcct gcacaggctt 60
g 61

<210> 16
<211> 61
<212> DNA
<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:U3 3' mutagenic
PCR primer for U3 constructs

<400> 16
gatttatgca tgacaatgag ctacctgctg attttccact tccatttcct gcacaggctt 60
g 61

<210> 17
<211> 67
<212> DNA
<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:L1 3' mutagenic
PCR primer for L1 constructs

<400> 17
gatttatgca tgacaatgag ctacctgac tcaacattcc taatgggtcca tttcctgcac 60
aggcttg 67

<210> 18
<211> 67
<212> DNA
<213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence:L2 3' mutagenic
 PCR primer for L2 constructs

<400> 18
 gatttatgca tgacaatgag ctaccttggtg cccataatcc taatgggtcca tttcctgcac 60
 aggcttg 67

<210> 19
 <211> 8
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence:matrix
 metalloproteinase (MMP) recognition cleavage site,
 MMP substrate octapeptide for L1 constructs

<400> 19
 Gly Pro Leu Gly Met Leu Ser Gln
 1 5

<210> 20
 <211> 8
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence:matrix
 metalloproteinase (MMP) recognition cleavage site,
 MMP substrate octapeptide for L2 constructs

<400> 20
 Gly Pro Leu Gly Leu Trp Ala Gln
 1 5

<210> 21
 <211> 6
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence:urokinase
 plasminogen activator (uPA) recognition cleavage
 site, uPA favorite sequence, uPA substrate
 hexapeptide for U2 constructs

<400> 21
 Gly Ser Gly Arg Ser Ala
 1 5

<210> 22
 <211> 6
 <212> PRT
 <213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:urokinase
plasminogen activator (uPA) recognition cleavage
site, uPA favorite sequence, uPA substrate
hexapeptide for U3 constructs

<400> 22

Gly Ser Gly Lys Ser Ala
1 5

<210> 23

<211> 6

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:tissue-type
plasminogen activator (tPA) recognition cleavage
site, tPA favorite sequence

<400> 23

Gln Arg Gly Arg Ser Ala
1 5

<210> 24

<211> 16

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:DTGM-WT furin
sensitive surface loop sequence

<400> 24

Cys Ala Gly Asn Arg Val Arg Arg Ser Val Gly Ser Ser Leu Ser Cys
1 5 10 15

<210> 25

<211> 16

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:DTGM-U2 surface
loop sequence cleaved by urokinase-type
plasminogen activator (uPA)

<400> 25

Cys Ala Gly Asn Gly Ser Gly Arg Ser Ala Gly Ser Ser Leu Ser Cys
1 5 10 15

<210> 26

<211> 16

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:DTGM-U3 surface
loop sequence cleaved by urokinase-type
plasminogen activator (uPA)

<400> 26

Cys Ala Gly Asn Gly Ser Gly Lys Ser Ala Gly Ser Ser Leu Ser Cys
1 5 10 15

<210> 27

<211> 18

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:DTGM-L1 surface
loop sequence cleaved by matrix metalloproteinase
(MMP)

<400> 27

Cys Ala Gly Asn Gly Pro Leu Gly Met Leu Ser Gln Gly Ser Ser Leu
1 5 10 15

Ser Cys

<210> 28

<211> 16

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:DTGM-Fu surface
loop sequence cleaved only by furin

<400> 28

Cys Ala Gly Asn Arg Ala Ala Arg Ser Val Gly Ser Ser Leu Ser Cys
1 5 10 15

<210> 29

<211> 9

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:plasminogen
activator cleavage site, uPA and tPA physiological
substrate sequence

<400> 29

Pro Cys Pro Gly Arg Val Val Gly Gly
1 5

<210> 30

<211> 6

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:Diphtheria
toxin (DT) cleavage sequence amino acids 163-170

<400> 30

Arg Val Arg Arg Ser Val

1

5